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Role of hydration water in dynamics of biological macromolecules

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Abstract

We present an overview of neutron scattering studies of the dynamics in hydrated and dry protein and RNA. We demonstrate that the difference observed in the dynamics of dry lysozyme and dry RNA is related to the methyl group contributions to the protein dynamics. Hydration of biological macromolecules leads to significant activation of conformational transitions. They appear in neutron scattering spectra as an additional slow relaxation process that exhibits a strongly stretched spectral shape and slightly non-Arrhenius temperature dependence of the characteristic relaxation time. Our analysis suggests that the appearance of this relaxation process in the experimentally accessible frequency window is the main cause for the sharp rise of the atomic mean-squared displacements (the so-called dynamic transition) that takes place in hydrated biomolecules at $T \approx 200$–220 K.

Keywords: Protein dynamics; RNA dynamics; Neutron scattering; Hydration water

1. Introduction

It is becoming increasingly obvious that understanding functions of biological macromolecules (proteins, RNA, DNA) requires understanding their dynamics, i.e. conformational changes and fluctuations. Knowledge of the structure of biomolecules is not sufficient for analysis of their activity; one needs to know various conformational states, energy barriers separating them and the rate of the conformational transitions. However, our knowledge of dynamics of proteins and other biological macromolecules remains rather limited.

Different techniques can be used for the analysis of protein dynamics. NMR provides detailed but very local information on motions of particular groups of atoms [1,2]. Various optical techniques, such as Raman, infra-red, optical absorption and fluorescence, also provide information on conformational changes and kinetics of some conformational transitions. However, all these techniques usually probe only particular groups of atoms and are not very efficient in studies of the global motions of proteins. Recently, dielectric spectroscopy has received increased attention due to the extremely broad frequency range it can access [3,4]. Dielectric spectroscopy, although very efficient in estimating characteristic relaxation times for processes that involve motions of charges and dipoles, provides no microscopic information on molecular motions.

Among other techniques, neutron scattering has a significant advantage because it measures atomic motions directly and provides information on local and global motions [5,6]. Hydrogen/deuterium exchange provides a unique opportunity to separate motions of different parts of a macromolecule due to the much larger (≈40 times) incoherent scattering cross-section of hydrogen. Another important advantage of neutron scattering spectroscopy is that spectra are measured as a function of scattering wave vector Q. The latter provides information on the

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amplitude of atomic displacements, their geometry and cooperativity (in the case of coherent scattering). This technique becomes even more powerful when it is combined with results from molecular dynamics (MD) simulations. This combination provides detailed microscopic pictures of molecular motions and is becoming increasingly popular for analyzing the dynamics of biological macromolecules. It also helps tremendously in adjusting the models and parameters used in MD simulations.

In this paper we present an overview of neutron scattering results obtained on protein lysozyme, RNA and DNA. We focus on the role of hydration water in promoting (enhancing) conformational fluctuations in biomolecules. We demonstrate that the main contribution to the relaxation spectra of dry proteins comes from the dynamics of methyl groups. The dynamics of dry RNA and DNA show more harmonic-like behavior due to the low content of methyl groups in these biomolecules. Hydration of all biomolecules leads to activation of an additional relaxation process. We show that the appearance of this process in the frequency range accessible to neutron spectroscopy is the main cause of the dynamic transition observed in hydrated biomolecules in the temperature range ≈200–220 K [7–10].

2. Experimental

Hen–egg white lysozyme (Sigma–Aldrich) was dialyzed to remove salts and then lyophilized. The protein powder was dissolved in D_2O to replace the exchangeable hydrogen atoms with deuterium atoms. A dry sample was obtained after lyophilization of the deuterium-exchanged protein and had a residual hydration level of $h \approx 0.05$ (g of water per gram of protein). A sample with $h \approx 0.4$ was prepared by isopiestic equilibration of the lyophilized powder with a saturated solution of K_2SO_4 in D_2O. The hydration levels were determined by thermogravimetric analysis (TGA), by measuring the mass change upon drying the samples. The weight of the samples before and after the experiments remained the same. The High Flux Back-scattering Spectrometer (HFBS) NG2 [11] was employed for analysis of the quasielastic scattering (QES) spectra of dry and wet lysozyme in the energy range $\Delta E = \pm 17$ μeV (≈4 GHz) and scattering wave vector range $0.25\ \text{Å}^{-1} < Q < 1.75\ \text{Å}^{-1}$. Neutron scattering data were corrected for background scattering and scattering from the sample cell, and analyzed using DAVE software provided by NIST [12]. The spectra were normalized by the mass of lysozyme in each sample. No correction for multiple scattering has been applied. Additionally, the data for lysozyme, RNA and DNA presented earlier in [10,13–15] were reanalyzed to emphasize general behavior in dynamics of biological macromolecules.

We want to emphasize that neutron spectroscopy probes essentially motions of hydrogen atoms due to their extremely high incoherent scattering cross-section [5]. The hydrogen atoms in the biomolecules contribute ~90% of the total scattering in the dry samples and ~80% of the total scattering in samples with hydration level $h \approx 0.4$ [14]. Because the hydrogen atoms are distributed evenly across the protein, RNA and DNA, their dynamics reflects the dynamics of the whole biomolecule.

3. Dynamics of dehydrated biomolecules

It is known that the dynamics of biological molecules and their activity are strongly suppressed in the dehydrated state. So, hydration water plays a very important role in the conformational transitions of biomolecules. However, before starting an analysis of hydrated biomolecules we want to focus on the dynamics of dry proteins and nucleic acids (RNA, DNA). One of the easiest quantities to measure in neutron scattering experiments is the mean-squared atomic displacement $\langle r^2 \rangle$. This is usually estimated from the Q-dependence of the elastic intensity [5,7,14]. Analysis of $\langle r^2 \rangle$ for dry lysozyme and RNA (Fig. 1) shows that there is a significant difference in behavior of these two biomolecules at temperatures above ≈100 K. The protein shows a much larger amplitude of $\langle r^2 \rangle$ than RNA.

However, $\langle r^2 \rangle$ is an integrated quantity that includes all kinds of motions (vibrations, rotations, conformational changes and diffusion) that occur on the time scale resolved by the spectrometer (≈2 nanosecond and shorter in our case). Analysis of the energy-resolved spectra and their Q-dependence usually helps to understand the various contributions to the observed $\langle r^2 \rangle$. Fig. 2 presents the dynamic structure factor $S(Q,v)$ of dry lysozyme and RNA. The spectra for the protein show strong quasielastic scattering that becomes visible already at $T = 150$ K, while no QES intensity is visible in the spectra of dry RNA even at $T = 300$ K (Fig. 2).

The QES contribution is an indication of a relaxation process that enters the experimental frequency window. Thus, the observed QES (Fig. 2) suggests that there is a strong relaxation contribution to the spectra of the dry

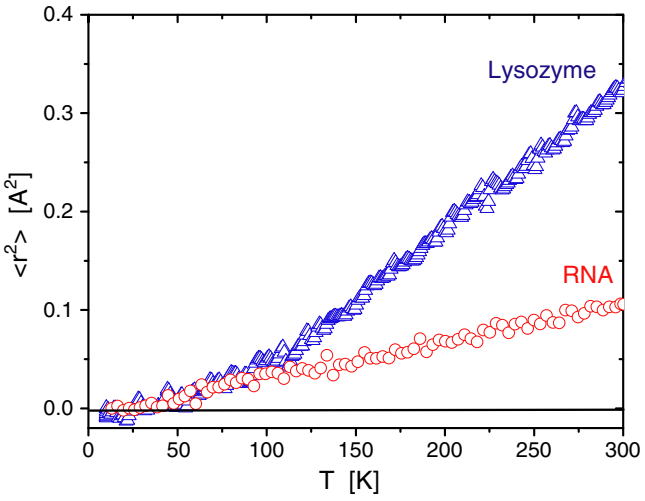

Fig. 1. Mean-squared atomic displacements in dry Lysozyme and RNA (data from [10]). Strong differences appears at $T > 100$ K.

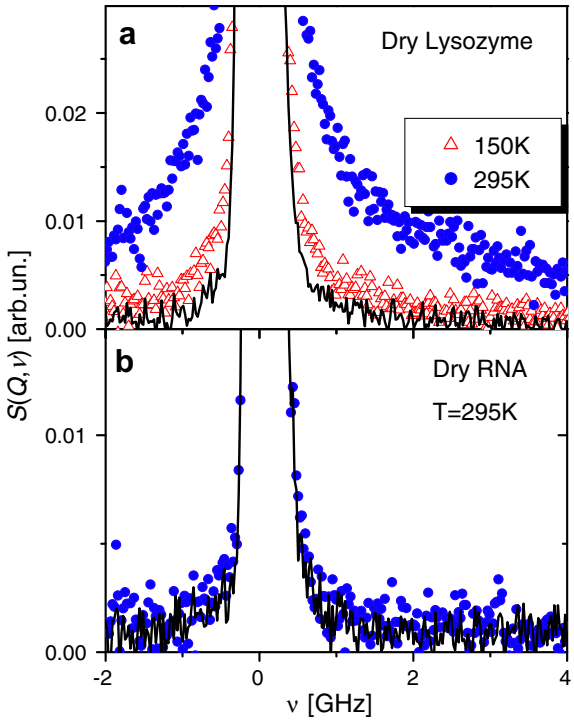

Fig. 2. Dynamic structure factor of dry lysozyme (a) and dry RNA (b). The solid lines represent the resolution function. The spectra are summed up over all Qs because no significant Q-dependence of the spectral width has been observed. Error bars are of the order of the data scattering.

protein. A tail of this relaxation appears in our experimental frequency window at very low T (Fig. 2a) and is the reason for the observed increase in $\langle r^2 \rangle$ of dry lysozyme at $T \approx 100$ K (Fig. 1). This contribution is absent in the spectra of dry RNA and might be the main reason for the different behavior of $\langle r^2 \rangle$ in these biomolecules (Fig. 1).

The QES contribution in dry proteins has been ascribed to methyl group rotation [13,14,16–18] because ~25% of all non-exchangeable hydrogen atoms (main contributors to the neutron scattering spectra) are methyl atoms. Moreover, methyl groups have rather low energy barriers for rotation and their dynamics should reach the nanosecond time scale already at low temperatures [2,13]. Detailed studies of the QES spectra, their temperature and Q-dependencies clearly demonstrate that methyl group dynamics is the main contributor to the QES spectra of dry lysozyme [13,14] and dry myoglobin [17,18]. This conclusion has been supported by detailed analysis of MD simulations [14]. Moreover, MD simulations provide a microscopic interpretation for experimentally observed broad distribution of relaxation times in methyl groups: Characteristic rotational times depend on the type of amino-acid residue and its position in the protein [14].

In contrast to proteins, RNA has negligible number of the methyl groups (less than 5% of all non-exchangeable hydrogen atoms). As a result, no significant QES intensity appears in the neutron scattering spectra in the measured frequency range (Fig. 2b). This result also explains the low $\langle r^2 \rangle$ observed for this biomolecule (Fig. 1). Earlier analysis of dry DNA molecules also reveals no significant QES contribution to the neutron scattering spectra even at $T = 315$ K [15]. The reason is the same – very low concentration of methyl groups in DNA.

Thus the onset of anharmonicity observed in dry proteins at $T \sim 100$ K is ascribed to the onset of methyl group rotations [14]. These results suggest that even dry proteins have significant anharmonic contributions in their dynamics. This observation contradicts the traditional view of harmonic behavior of proteins in the dehydrated state. What is more important, the results suggest that one cannot use the measured $\langle r^2 \rangle$ in proteins as estimates of their "effective" elastic constants. The idea of these estimates was strongly promoted in the recent publication [19]. The analysis presented in [14] demonstrates, however, that about third of the observed $\langle r^2 \rangle$ in proteins is due to contributions from methyl group rotations. This contribution does not reflect the "elasticity" of the protein.

4. Dynamic transition in hydrated biomolecules

Hydration of biological macromolecules at sufficiently high level (usually at $h > 0.2$) strongly enhances their activity and dynamics. $\langle r^2 \rangle$ in hydrated biomolecules exhibits a sharp change at $T_D \sim 200$–220 K (Fig. 3) [7–10]. This change indicates activation of some motions and is traditionally called the dynamic transition [6,7]. It is important to note that the dynamic transition appears around the same temperature range for all hydrated proteins and also for hydrated RNA [10] and DNA [15] (Fig. 3). Thus the dynamic transition temperature T_D is essentially the same for biological macromolecules regardless of their structure and backbone chemistry (amino-acids or nucleic acids).

This observation leads to speculations that the solvent (water of hydration) may be the main reason for the observed dynamic transition [9]. The dynamics of

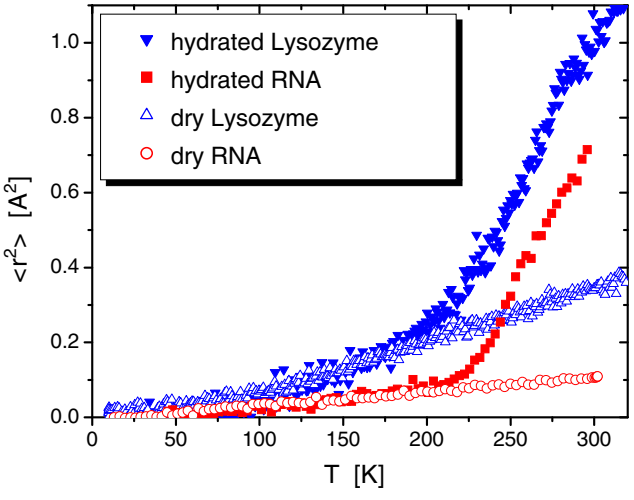

Fig. 3. Mean-squared atomic displacements in dry and wet lysozyme and RNA (data from [10]). The sharp rise in $\langle r^2 \rangle$ of hydrated biomolecules at T above 200 K is traditionally called the dynamic transition.

biomolecules are strongly coupled to the dynamics of water and this might result in some universal behavior in the dynamics of hydrated proteins, RNA and DNA. This idea has been supported by the analysis of proteins placed in non-aqueous solutions: The dynamic transition is shifted to higher temperatures for proteins placed in glycerol [8,20] and is suppressed at least up to $T \sim 330$ K for proteins placed in trehalose [20,21]. Thus solvents clearly play an important role in the dynamic transition of biomolecules.

Additional arguments emphasizing the crucial role of solvents in this transition, come from simulations. Smith and co-workers applied the dual heat bath method to the analysis of the dynamic transition in hydrated myoglobin [22]. In this approach one can vary the temperature of the solvent (water) and the protein independently. The authors demonstrate that the hydrated protein does not undergo the dynamic transition up to 300 K when the temperature of the surrounding water is held constant. However, if the protein temperature is fixed at 300 K then a dynamic transition in the protein occurs when the solvent temperature crosses 200 K [22]. Tarek and Tobias performed another simulation experiment: They artificially restricted the translational motion of water molecules [23]. The dynamics of ribonuclease A in these conditions (no restrictions were imposed on protein motions) resembled that of the dehydrated protein. Thus, restricting translation of the solvent molecules causes the hydrated protein to behave as if it is below the dynamic transition even at $T = 300$ K.

5. Temperature dependence of structural relaxation in wet Lysozyme

Although the role of the solvent in the dynamic transition of biological macromolecules seems to be well established, the nature of the dynamic transition and what is really happening to the protein at T_D remains the subject of active discussions [1,6,9,19–26]. Analysis of $\langle r^2 \rangle$ provides very limited information. Analysis of energy-resolved spectra, their Q and temperature dependence is significantly more informative. Fig. 4 compares QES spectra of wet and dry biomolecules at different temperatures. The QES spectra of dry and wet lysozyme are the same at $T = 150$ K (below T_D, not shown) suggesting that the same relaxation process is active in both states of the molecule. QES spectra of wet lysozyme becomes slightly broader than in the dry protein at $T = 200$ K ($\sim T_D$) and the difference increases dramatically at $T = 295$ K (much above T_D) (Fig. 4a). These results indicate an additional relaxation process in wet lysozyme that enters our experimental frequency window at temperatures around T_D. For simplicity we will call this process the "slow" relaxation process. The situation is more obvious in the case of RNA because the dehydrated molecule does not show any significant QES contribution even at ambient temperature (Fig. 2b). There is no significant QES intensity at $T = 200$ K ($\sim T_D$) in wet

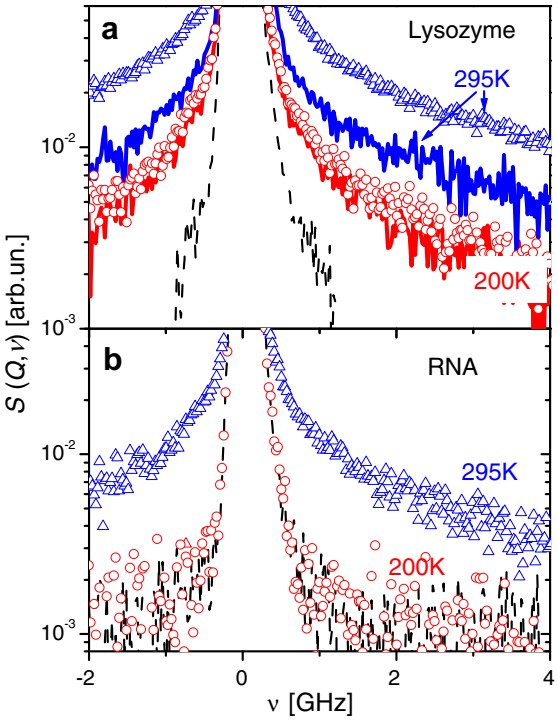

Fig. 4. Dynamic structure factor of dry (lines) and wet (symbols) lysozyme (a) and wet RNA (b) (RNA data from [10]). The dashed lines represent the resolution function. The spectra are summed up over all Qs because no significant Q-dependence of the spectral width has been observed.

RNA, while significant QES intensity appears in the spectra of wet RNA at $T = 295$ K (much above T_D). Similar observations have been reported also for DNA [15].

Thus, the observed sharp change in the temperature dependence of $\langle r^2 \rangle$ at T_D is caused by a slow relaxation process that enters the experimental frequency window in this temperature range. In order to analyze the spectra of the slow relaxation process we present the neutron scattering data as an imaginary part of susceptibility $\chi''(Q, \nu) \propto S(Q, \nu)/n(\nu)$, where $n(\nu) = [\exp(h\nu/kT) - 1]^{-1}$ is the temperature Bose factor, (Fig. 5). Dynamic susceptibility describes the response of the system to thermal fluctuations or to some external perturbations. Its imaginary part reflects the relaxation (loss) spectra of the system similar to the dynamic structure factor traditionally used for description of the scattering data. The susceptibility presentation of the scattering data, however, has a number of advantages [14]: (i) the trivial temperature dependence (Bose factor) is removed; (ii) the scattering data can be directly compared to mechanical $G''(\nu)$ and dielectric $\varepsilon''(\nu)$ loss data; (iii) any relaxation process appears as a maximum with characteristic relaxation time $\tau \sim (2\pi\nu_{max})^{-1}$ and the spectral shape of the maximum provides a direct estimate of the stretching of the relaxation process. In order to expand the frequency range we included earlier neutron scattering data [14] obtained with a time-of-flight spectrometer. We want to emphasize that the susceptibility presentation significantly enhances the background and noise at the higher frequency

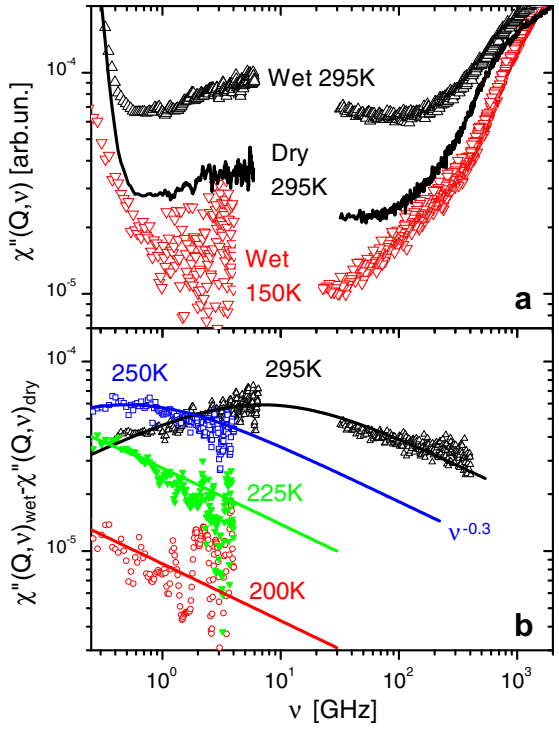

Fig. 5. (a) Imaginary part of the susceptibility spectra of wet and dry lysozyme. The spectra combine back-scattering data with earlier time-of-flight data from [14]. (b) The difference between the susceptibility spectra of wet and dry lysozyme at different temperatures (symbols). The lines are fits with a stretched function with the stretching parameter $\chi''(\nu) \propto \nu^{-0.3}$.

end of the measured spectra and it is not very efficient when the signal is weak. Good illustration of this point is the spectrum of wet lysozyme measured at $T = 150$ K (Fig. 5a).

The susceptibility spectrum of wet lysozyme at $T = 150$ K shows only fast dynamics that dominates at frequencies above ~20 GHz and the tail of some slower relaxation process visible at $\nu < 2$ GHz (Fig. 5a). This is the high-frequency tail of methyl group contribution that was discussed in Section 3. The same methyl group rotation dominates the spectra of dry lysozyme at $T = 295$ K and appears as a broad maximum at frequencies in the range ~2 GHz to 6 GHz (Fig. 5a). The large width of the relaxation spectrum in dry lysozyme is ascribed to a broad distribution of energy barriers in methyl groups [2,14].

A much stronger relaxation contribution appears in the spectrum of hydrated lysozyme at $T = 295$ K (Fig. 5a). Earlier analysis of MD simulations results demonstrates that methyl relaxations are essentially independent of the hydration of the protein [14]. So, the observed increase in the QES intensity must be related to the additional slow relaxation process. In order to analyze the spectrum of the slow relaxation process, we subtracted the spectra of the dry sample from the spectra of the hydrated sample. In this way we essentially remove the contribution from the methyl groups. The so-obtained spectra exhibit a very broad symmetrically stretched peak with extremely high stretching $c''(\nu) \propto \nu^{-b}$, $b \approx 0.3 \pm 0.05$ (Fig. 5b). The frequency of this peak decreases with decreasing temperature, reflecting an increase in relaxation time. Only the high-frequency tail of the process is observed in the experimentally accessible frequency window at $T < 250$ K (Fig. 5b). Assuming that the shape (stretching and amplitude) of this process does not change significantly with temperature, we estimated its relaxation time at $T < 250$ K from the fit of the high frequency tail (Fig. 5b).

The relaxation time of this process shows a slightly non-Arrhenius temperature dependence (Fig. 6a). In addition, the dashed line indicates the relaxation time expected for methyl groups. The results illustrate that analysis of neutron scattering spectra of hydrated lysozyme by a single relaxation process will provide misleading results: The spectra are dominated by the slow process at higher temperatures and by methyl group rotations at lower temperatures, $T < 250$ K, (Figs. 4a and 5a). Thus the estimated relaxation time will be a combination of characteristic times for the two relaxation processes. One needs to take into account the contribution from the methyl groups explicitly in order to estimate the relaxation time of the slow process. The dotted line in Fig. 6a indicates the resolution of the back-scattering spectrometer used in our measurements of $\langle r^2 \rangle$. It shows very clearly that the appearance of the slow relaxation process in our experimental frequency window is the main cause for the observed sharp rise in $\langle r^2 \rangle$ of hydrated lysozyme.

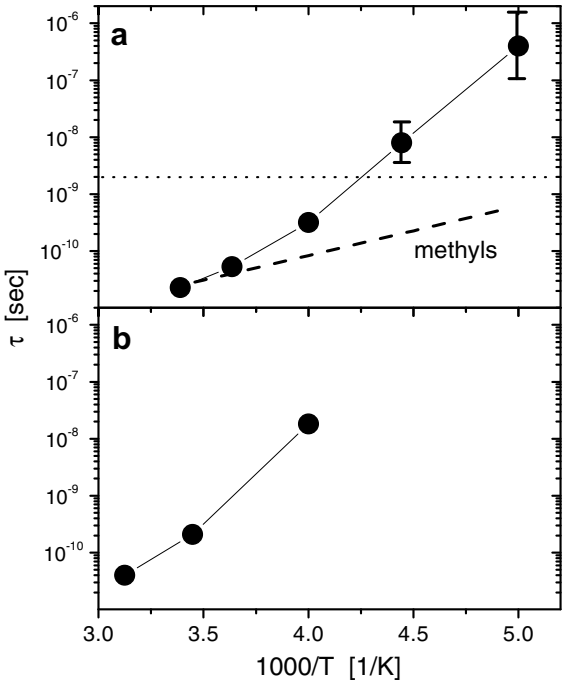

Fig. 6. (a) Temperature dependence of the characteristic relaxation time of the slow process in hydrated lysozyme. Use of the low-frequency tail of the peak results in large error bars for estimates of τ at $T < 250$ K. The dashed line represents the temperature dependence of the relaxation time of the methyl groups (data from [14]). The dotted line indicates the resolution of the spectrometer. (b) Temperature dependence of relaxation times in hydrated DNA (data from [20]).

As we discussed above, the situation is simpler in the case of RNA and DNA because the methyl group contribution to the neutron scattering spectra of nucleic acids is negligible. The estimation of the characteristic relaxation time of the slow process in DNA was done earlier in [20] and is presented in Fig. 6b. It also shows slightly non-Arrhenius temperature dependence and also reaches the experimental frequency window at similar temperature range.

We want to emphasize that the idea of relating the dynamic transition to a relaxation process entering the experimentally accessible frequency window has been discussed in many earlier papers [20,24–27]. In particular, Smith and co-workers presented some experimental and simulation results supporting this idea [24,25]. Using two spectrometers, IN16 with resolution corresponding to characteristic time ~5 ns and IN6 with characteristic time ~0.1 ns, they demonstrate that the temperature at which $\langle r^2 \rangle$ shows a strong increase depends on the spectrometer resolution. Frauenfelder and co-workers also tried to justify this idea from analysis of $\langle r^2 \rangle$ [26]. Dirama et al. demonstrated the same result in simulations of lysozyme in glycerol [27]. In that case, glycerol imposes higher friction on the protein and slows down its dynamics relative to the dynamics of hydrated protein. As a result, the slow process enters the accessible frequency window at a higher temperature and the dynamic transition temperature measured from $\langle r^2 \rangle$ of protein placed in glycerol shifts to $T \sim 270$–300 K [8,20,27]. Thus, there is growing experimental and simulation evidence that nothing special happens to biological macromolecules around the dynamic transition temperature and the observed sharp rise in $\langle r^2 \rangle$ is related to a slow relaxation process that enters the accessible frequency window around this temperature range.

The microscopic nature of the slow relaxation process remains unclear. Analysis of the Q-dependence of the QES spectra in wet lysozyme indicates that the process involves a significant part of the protein atoms and has an amplitude of atomic displacements of ~3 Å. So, it represents some global protein relaxation. Normal mode analysis performed by Tournier and Smith [28] suggested that rigid body motions of secondary structures are the modes activated above the dynamic transition temperature. So, one microscopic interpretation is that the slow process might be due to motions of secondary structures. More detailed neutron scattering and MD simulations studies are necessary to gain a better understanding of the microscopic nature of the slow relaxation process and extremely strong broadening of its spectra (Fig. 5b).

6. Conclusions

We demonstrate that the dynamics of dehydrated proteins exhibits significant relaxation that is ascribed to methyl group rotations. The dynamics of RNA appears much more harmonic because of the low concentration of methyl groups in this biomolecule. We want to emphasize that any analysis of neutron scattering spectra of proteins should explicitly take methyl group rotations into account. It might be also useful for the analysis of MD simulation results. As has been shown in [27,29], excluding the contribution from the methyl groups makes the analysis and interpretation of simulations data much clearer.

Hydration of biological macromolecules leads to the activation of an additional relaxation process. We emphasize that the dynamic transition appears in proteins, RNA and DNA at the same temperature range, i.e. it appears to be independent of molecular structure and backbone chemistry. These observations together with additional evidence from experiments and simulations suggest that hydration water controls the observed dynamic transition. Detailed studies of relaxation spectra demonstrate that an additional slow relaxation process appears in the experimental frequency window at temperatures above the dynamic transition. This process is extremely stretched indicating either a broad distribution of relaxation times or a very complex relaxation process. Its characteristic relaxation time shows a slightly non-Arrhenius temperature dependence. Our analysis of the experimental spectra suggests that the dynamic transition observed as a sharp rise of $\langle r^2 \rangle$ is caused by the appearance of this slow process in the experimentally accessible frequency window.

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